

to the capillary wall thereby altering the zeta potential and resulting electro-osmotic flow, such as charged proteins. Samples such as blood specimens, which contain interfering components may require the use of surface-modified capillaries to prevent adsorption. Alternatively, sample preparation steps may be used to remove the interfering matrix components.

The capillary electrophoresis method of the invention was compared to a conventional spectrophotometric method of ethanol analysis. Ten samples containing between 0 and 4 g/L of ethanol were analyzed by both the capillary electrophoresis assay and the Sigma Diagnostics Alcohol kit. The Sigma spectrophotometric method employs the same enzymatic reaction as the capillary electrophoresis assay. After ten minutes of incubation of the sample in pH 9.0 buffer containing ADH, NAD, and hydrazine trapping agent, the increase in absorbance at 340 nm is directly proportional to alcohol concentration in the sample. FIG. 14 compares the results of the two assays. Linear regression analysis of the data yields $y(\text{Sigma}) = 1.04 \times (\text{capillary electrophoresis}) - 0.038 \text{ g/L}$, with a correlation coefficient of 0.995. The paired results of the eight samples yielded a paired-t calculation of $t_{calc} = 0.284$ compared to a table value of $t_{0.950}$ of 2.365. These results indicate that the two methods yield similar values for the determination of ethanol. The reproducibility of the capillary electrophoresis method is also comparable to that of the Sigma assay (4.1% and 1.08% relative standard deviations in two ethanol spiked serum pools).

The specificity of the assay is consistent with that observed with all ADH assays. While ADH does not react appreciable with methanol or acetone, it does react with certain alcohols other than ethanol. The rate of oxidation of these alcohols decreases in the following order: ethanol > allyl alcohol > n-propanol > n-butanol > n-amyl alcohol > isopropanol. Although this lack of specificity is a disadvantage for all ADH-based assays, isopropanol is the only interfering alcohol which is encountered clinically. For the conditions previously described (pH 9, 60 μm inner diameter uncoated capillary, 25 cm separation length, 100 V/cm), the flow rate of buffer/reagent solution is approximately 0.38 $\mu\text{L}/\text{min}$. A typical 16 minute assay requires only

about 6 μL of buffer/reagent solution containing about 5 nanomoles of NAD and 0.5 units of ADH.

Other embodiments are within the following claims.

We claim:

1. A method of analysis of an analyte in a sample, the method comprising:

- (a) introducing first and second reactants and a sample comprising an analyte into a capillary containing an electrophoretic running buffer, wherein chemical contact between said analyte and said first reactant results in the breaking or formation of a covalent bond in said analyte to produce a first product, and wherein chemical contact between said first product and said second reactant results in the breaking or formation of a covalent bond in said first product to produce a detectable second product, and wherein said analyte and said first product or said reactants are electrically charged;
- (b) imposing along the length of said capillary an electric potential for a time sufficient to bring into chemical contact by electrophoretic mobility within said capillary said analyte and said first reactant so as to induce formation of said first product, and then said first product and said second reactant to produce said second detectable product; and
- (c) detecting said second product.

2. The method of claim 1 wherein said capillary used in step (a) contains said first and second reactants loaded thereinto prior to the introduction of said sample.

3. The method of claim 1 wherein said electric potential is imposed on said capillary for a time sufficient to separate said second product from said analyte or said reactants prior to step (c).

4. The method of claim 1 wherein said electric potential is imposed on said capillary for a time sufficient to separate by electrophoretic motion said analyte from other sample components prior to said chemical contact between said analyte and said first reactant.

5. The method of claim 1 wherein said analyte and said first product comprise an enzyme substrate and said first and second reactants each comprise an enzyme.

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